Cytotoxicity of sequiterpene lactones derived from higher plants

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Summary

The aim of the experiments described in thesis was to investigate the in-vitro cytotoxicity of two groups of sesquiterpene lactones (SQLs) towards tumour cells. One group of SQLs containing Michael-addition sites (MAS), and another group having endoperoxide moieties. MAS-containing compounds are known to bind to free sulphydryl groups on proteins. This mechanism explains the cytotoxic action towards cells. The endoperoxide bridge-containing artemisinin and its derivatives are used to treat malaria. Reactive degradation products are thought to kill malaria parasites (see **Chapter 1**). Based on this potentially reactive structure, we investigated the cytotoxicity of artemisinin and derivatives towards tumour cells, with the ultimate goal to obtain a "lead" for new anticancer compounds against tumours in humans.

Chapter 2 describes two methods for measuring cytotoxicity. The cytotoxicity of artemisinin and some derivatives to a murine Ehrlich ascites tumour cells and a human HeLa S3 cancer cell line was determined using the MTT and the clonogenic assay. The MTT assay cannot distinguish between growth inhibition and cell killing, while the clonogenic assay detects actual cell death. The use of both assays to test a certain compound may provide information on the mode of its cytotoxicity (*i.e.* growth inhibition versus cell killing). The endoperoxides artemisinin and the dimer of dihydroartemisinin showed much higher cytotoxicity in the MTT assay as compared to the clonogenic assay. Thus, these compounds mainly induced growth inhibition. Artemisitene and eupatoriopicrin, which possess MAS, are able to kill cells. Both tests yielded comparable results. For the reference compounds cisplatin and doxorubicin the MTT assay showed lower cytotoxicity than the clonogenic assay. This may be explained by "delayed" cell death. The duration of the MTT assay is shorter than that of the clonogenic assay. Cells that will not form a colony in the clonogenic assay may still convert MTT.

Chapter 3 deals with SQLs with MAS. The study describes the cytotoxicity of 21 helenanolide-type SQLs to an Ehrlich ascites tumour cell clone (EN2). Structure-

cytotoxicity relationships were determined with respect to the three-dimensional structures of the compounds as visualized by molecular modelling software. We demonstrated that steric effects might be an important factor for the extent of cytotoxicity of MAS-containing SQLs.

Chapter 4 contains a report on the investigation of the stability of artemisinin in the aqueous environment of the in-vitro tumour-cell system (RPMI 1640/10% Foetal Bovine Serum) with reference to its cytotoxic action. Literature data show that artemisinin can react with Fe^{2+} yielding reactive intermediates leaving artemisinin G as a major end-product. Our study showed that only a large concentration of Fe^{2+} reacted with artemisinin in distilled water, phophate-buffered saline (PBS), and RPMI/FBS cell culture medium (24 h of incubation). Artemisinin was degraded, whereas artemisinin G was formed. HPLC analysis showed 100% recovery of artemisinin in absence of Fe^{2+} in distilled water, and RPMI/FBS with or without EN2 cells at 37°C for at least 24h. Artemisinin G could not be detected. In addition, incubation of artemisinin in RPMI/FBS with or without cells at 37°C for 24h prior to a cytotoxicity assay did not significantly change its cytotoxic action. Based on these results, we stated that the cytotoxicity to tumour cells was caused by unchanged artemisinin.

In **Chapter 5**, we described the cytotoxicity of some artemisinin derivatives against EN2 tumour cells using the MTT assay. Artemisinin was clearly more cytotoxic than deoxyartemisinin, which lacks the endoperoxide bridge. Ether-linked dimers of dihydroartemisinin with defined stereochemistry were found to differ in the extent of the cytotoxic effect on EN2 cells. The nonsymmetrical dimer was more cytotoxic than the symmetrical dimer. The nonsymmetrical dimer of dihydrodeoxyartemisinin lacking the endoperoxide bridges was also effective in the MTT assay, although less cytotoxic than the dimers of dihydroartemisinin. Similarly, the symmetrical dimer was less effective than the nonsymmetrical. The results suggested that the endoperoxide bridge was not crucial for cytotoxicity to the tumour cells, but contributed to the cytotoxic effect associated with the ether linkage of the dimers. Flow cytometry data indicated that the dimers of dihydroartemisinin caused an accumulation of the cells in the G_1-phase of the cell cycle. In contrast, artemisinin only caused a slight increase of S-phase cells.

With respect to their cytotoxicity towards Ehrlich ascites tumour cells, the dimers of dihydroartemisinin may be potential antitumour compounds. An antitumour agent should not be strongly active to normal cells. **Chapter 6** describes the in-vitro cytotoxic activity of artemisinin and some derivatives against murine bone marrow. This was determined using a clonogenic assay for committed progenitor cells of the granulocyte-monocyte lineage (CFU-GM assay). Similar to the cytotoxicity to tumour cells nonsymmetrical dimers were more toxic than symmetrical dimers. Although artemisinin was more cytotoxic than deoxyartemisinin, the nonsymmetrical dimer of dihydroartemisinin and the corresponding endoperoxide-lacking dimer of dihydrodeoxyartemisinin were equally cytotoxic to CFU-GM cells. Unfortunately, most compounds displayed higher cytotoxicity to CFU-GM cells than to a cloned Ehrlich

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Chapter 7
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ascites tumour cell line. According to the these in-vitro data, the compounds were more cytotoxic to normal cells than to tumour cells. However, in-vivo testing might have a different outcome.

The dimers of dihydroartemisinin were subjected to the National Cancer Institute drug-screening programme consisting of about sixty human cancer cell lines derived from nine different tissues. Throughout the whole screen the nonsymmetrical dimer was more cytotoxic than the symmetrical dimer. The NCI selected our compounds for further in-vivo testing in mice.

Chapter 7 describes the possibilities for future research. Because the G_1 accumulation caused by the dimers of dihydroartemisinin was the most interesting finding of this thesis, the discussion focusses on the cell cycle to identify molecular targets.